THE EFFECT OF SOME FOOD HORMONES AND GLANDULAR PRODUCTS ON THE RATE OF GROWTH OF PARAMECIUM CAUDATUM.[1]

MARY H. CHAMBERS.

The animal body is adjusted to live upon plant or animal tissue. It is generally accepted now that the adequate diet must contain more than protein, carbohydrate, and fat, namely, an accessory factor or vitamin. Recent investigations upon dietaries of infants have shown the importance of some of the accessory factors. Hopkins (1912) added yeast to a diet of purified food stuffs and found that it accelerated the growth of rats. The same result was obtained later by Funk and Macallum (1915). More recently Osborne and Mendel (1917) have shown that the addition of yeast to the ration not only causes an increase in rate of growth of rats, but it acts as a stimulus to the appetite, and when removed from the diet growth ceases and decline ultimately follows. As yeast supplies a definite substance for growth so also potato juice acts as a corrective in cases of malnutrition. With these results in mind a series of experiments was undertaken to determine whether or not extracts of yeast and potato juice added to the diet of *Paramecium* produced any marked effect on the rate of division which according to Calkins (1902) is the index to vitality. The investigations were carried on under the direction of Dr. Florence Peebles, to whom the writer is indebted for suggestions and assistance.

A first set of experiments was made upon a pure line of *Paramecia* which had been maintained for several months on a diet of malted milk.

The solutions of yeast and potato which were employed were 1 per cent., and were made with distilled water. There were two methods of preparation with each. According to the first method the compressed yeast cake was merely dissolved in water, in the second, the yeast was first ground in order to allow

[1] From the Biological Laboratory, Bryn Mawr College.

the contents of the cells to enter the solution. One potato solution was boiled and strained, the other was strained raw. The controls were maintained in .2 per cent. solution malted milk. It was customary to feed the control and experimental lines an equal quantity of freshly made solutions every other day. The same food pipette was always used. Isolating pipettes were sterilized in boiling water each time used to avoid contamination in change of food and to kill any *Paramecia* that might have remained in the pipette. Such sterilization is not sufficient to destroy bacteria. All *Paramecia* were kept in depression slides in a moist chamber at constant temperature.

I. Experiments with Food Hormones.

The following tables show the number of divisions in several control and experimental lines. This experiment was carried on for twelve days and a record kept of each daily division. The table shows the data averaged in periods of six days each. Varying proportions of food substances were used. Although the total quantity of food was always the same in both lines, one set of experiments contained two drops of raw potato solution and one drop of the basic food substance—malted milk—with a control of three drops of malted milk;—and another—three drops of raw potato solution with a control of four drops malted milk.

Table I.

Potato (Raw). Daily Division Average For Six Day Periods.

Period.	Control.	Potato, Two Drops.	Control.	Potato, Three Drops.
1	5.5	7.5	4	7
2	8	6	5.5	7.5
Total	13.5	13.5	9.5	14.5
Average	1.11	1.11	.78	1.2

With raw potato the average rate of growth in the weaker solution was practically the same as that of the control while the rate of division for the stronger extract exceeded that of the control.

Table II.

Potato (Boiled). Daily Division Average For Six Day Periods.

Period.	Control.	Potato, Two Drops.	Control.	Potato, Three Drops.
1 2	6.5 6	4 8.5	7 6	7 5.5
Total	12.5	12.5	13	12.5
Average	1.03	1.03	1.08	1.03

It will be observed that when the potato extract was boiled the average rate of division was about the same for the weaker and stronger solutions.

A similar experiment was carried on at the same time with yeast. Two and three drops each of the whole and ground yeast were used.

Table III.

Yeast (Whole). Daily Division Average For Six Day Periods.

Period.	Control.	Yeast, Two Drops.	Control.	Yeast, Three Drops.
1 2	7 4	6 7	4.5 3.5	7 8.5
Total	11	13	8	15.5
Average	.91	1.08	.66	1.28

As this table shows, the individuals in the solution containing whole yeast divided more rapidly than the control.

Table IV.

Yeast (Ground). Daily Division Average For Six Day Periods.

Period.	Control.	Yeast, Two Drops.	Control.	Yeast, Three Drops.
1 2	.3 0	7.5 9	6 4.5	5 8.5
Total	3	16.5	10.5	13.5
Average	1.5	1.37	0.86	1.11

In this case a decided increase in the rate of division is shown when ground yeast is added to the diet.

A second set of experiments was made upon four races of *Paramecia* as follows: Race A had been in spring water in the

laboratory for several months; race B came from a culture procured from the University of Pennsylvania; race C came from Powers and Powers, Nebraska; race D, was a wild race recently obtained from a pond in the neighborhood of the college laboratory.

A comparative study of pure lines from these four races was carried on with the addition of yeast both whole and ground to the food substance. Two drops each were used. Malted milk remained the basic food substance.

Table V.

A. Laboratory Culture in Spring Water. Daily Division Average For Six Day Periods.

Period.	Control.	Yeast, Whole.	Control.	Yeast, Ground.
1	16	11.5	9.5	11
2	10	10	9.5	13.5
Total	26	21.5	19	24.5
Average	2.6	1.78	1.41	2.03

These individuals had previously undergone a period of starvation living in the laboratory under adverse conditions. Here the malted milk alone seemed more favorable to growth without the addition of whole yeast. It will be observed, however, that when ground yeast was added that there was a distinctly marked increase in the division rate.

Table VI.

B. Laboratory Culture in Hay Infusion. Daily Division Average For Six Day Periods.

Period.	Control.	Yeast, Whole.	Control.	Yeast, Ground.
1	12.5	10	10.5	10
2	13	10.5	8.5	14
Total	25.5	20.5	19	24
Average	2.11	1.7	1.6	2

The same result may be seen as in Table V., where the *Paramecia* had been in hay infusion.

Table VII.

C. Mixed Culture, Powers and Powers. Daily Division Averaged for Six Day Periods.

Period.	Control.	Yeast, Whole.	Control.	Yeast, Ground.
1	6.5	7	10	10.5
2	7	7	6	3
Total	13.5	14	16	13.5
Average	1.11	1.16	1.33	1.11

This was a rich culture containing many infusoria, and a high percentage of bacteria. In these individuals the rate showed little increase above the average.

Table VIII.

D. Wild Race. Daily Division Averaged For Six Day Periods.

Period.	Control.	Yeast, Whole.	Control.	Yeast, Ground.
1	9	5	11	7.5
2	12	2.5	8	1.5
Total	21	7.5	19	9
Average	1.75	.61	1.14	.75

The wild race of *Paramecia* had to undergo the greatest change. It thrived as the others on the rich diet of malted milk, but the experimental lines decreased exceedingly in division rate.

In the comparative study of these races it must be concluded that, (1) yeast stimulates growth as indicated by the rate of division, and (2) ground yeast is more favorable as food than whole yeast.

The physiological condition of the individual must be taken into consideration. For example in races A and B there was a higher rate of division than in races C and D. The latter ones had been in rich cultures before they were subjected to the experiment. Such a series also reveals the great variation in the rate for different individuals under the control conditions. For example, in Table V the variation of the control is 4.5, in Table VI., 3.2, in Table VII., 1.3 and Table VIII., 0.34. Although every effort was made to keep the conditions the same there is evidence of great variation.

In conclusion, the experiments performed with food hormones indicate (1) that potato extract will serve as a food but not as a growth accelerator, (2) that yeast extract serves as a food and stimulates growth, and (3) that the physiological condition of the individual plays a large part in determining the rate of division under experimental conditions. Results indicate that when food supply has been reduced a change of diet causes rapid increase in rate. Where food has been plentiful the rate of division has not been greatly increased.

2. Experiments with Glandular Diet.

Shumway (1917) has recently made a series of experiments with gland extracts. His controls were kept in a suspension of hay infusion. When adrenalin and pituitary extract were added these lines divided more than twice as rapidly as the control and with very little difference between them. In another experiment he determined to try what he termed a more crucial test,—*i. e.*, keeping the experimental lines in gland suspension alone without the basic food substance of hay infusion. Both lines died after a few days treatment.

For the following experiments a wild race of *Paramecia* was collected in January, 1918. Individuals descended from one original animal were put in hay infusion. On February 9, these *Paramecia* as control lines, were isolated in depression slides in a few drops of hay infusion and put in the moist chamber. Within twenty-four hours the sister *Paramecia* were isolated and the experimental lines established. The individuals were fed on solutions made from the tablets prepared in the laboratory of Armour & Co. The pituitary tablets were made from the desiccated gland of an ox and suprarenal from the desiccated gland of a sheep. The strength of the solution of the pituitary gland was .35 per cent., that of the suprarenal .36 per cent., being slightly stronger.

The experiments as recorded in Table IX. and Table X. were carried on for five weeks. With the pituitary solutions three proportions of food were used—first, control line two drops of hay infusion, experimental line one drop of hay infusion and one drop pituitary, second, control line three drops of hay infusion,

experimental line one drop of hay infusion and two drops of pituitary; third, control line four drops of hay infusion,—experimental line one drop of hay infusion and three drops of pituitary.

TABLE IX.

PITUITARY AND HAY INFUSION. Daily Division Average For Six-Day Periods.

Period.	Control.	Pituitary, One Drop.	Control.	Pituitary, Two Drops.	Control.	Pituitary, Three Drops.
1	.5	.66	.83	1.08	.5	.83
2	.5	1.0	.5	1.2	.92	1.0
3	.0	1.0	.83	.5	.66	1.3
4	.33	.75	.83	.5	1.2	.0
5	.5	1.0	.0	.25	.0	.33
Total	1.83	4.41	2.99	3.53	3.28	3.46
Average	.36	.88	.59	.65	.65	.69

There is an increase in division rate of experimental over control lines, but the amount of increase diminishes with an increasing amount of pituitary as food.

With the suprarenal two proportions of food were used;—first, control line two drops of hay infusion, experimental line one drop hay infusion, and one drop of suprarenal; second, control line three drops of hay infusion, experimental line one drop of hay infusion and two drops of suprarenal.

TABLE X.

SUPRARENAL AND HAY INFUSION. Daily Division Averaged For Six-Day Periods.

Period.	Control.	Suprarenal, One Drop	Control.	Suprarenal, Two Drops.
1	.8	1.0	1.5	1.1
2	.75	1.6	.66	1.1
3	1	.58	.0	1.0
4	1	.83	.66	1.2
5	.5	.66	.25	.41
Total	4.05	4.67	3.07	4.81
Average	.72	.943	.76	.96

These results are more constant than those obtained by feeding pituitary solution. There is a slight but decided increase over control lines.

The control of the experimental line containing two drops of suprarenal died and it was necessary to supply from stock which was kept in hay infusion.

A series of experiments was tried with mixtures of pituitary and suprarenal solutions in the basic food substance. The results indicate that the rate of division is greatly reduced. Sometimes the *Paramecia* would exist for seven days without dividing.

At this point in the work the control culture medium was changed from hay infusion to .2 per cent. malted milk. Another pedigreed race was started which had been in malted milk for several months. The solutions used were in two proportions—first, control line two drops of malted milk, experimental line one drop of malted milk, one drop of pituitary; second, control line three drops of malted milk, experimental line one drop of malted milk, and two drops of pituitary.

TABLE XI.

PITUITARY AND MALTED MILK SOLUTIONS. Daily Division Averaged for Nine Days.

Period.	Control.	Suprarenal, One Drop.	Control.	Suprarenal, Two Drops.
1	.89	0	1.1	0

In both experimental lines the *Paramecia* died after swimming about normally for five days without dividing. In no way can these results be correlated with the previous similar experiment where the basic food was hay infusion.

Solutions of suprarenal were the same as those above for pituitary.

TABLE XII.

SUPRARENAL AND MALTED MILK SOLUTION. Daily Division Average for Nine Days.

Period.	Control.	Suprarenal, One Drop.	Control.	Suprarenal, Two Drops.
1	.83	1	.77	1.1

There is an increase in division rate when Paramecia are fed suprarenal solution whether the basic fluid is hay infusion or malted milk.

CONCLUSIONS.

As a food hormone, potato extract apparently has little effect on the division rate of *Paramecium*. The influence of yeast is evident as exemplified in Tables III. and IV., *i. e.*, it increases the division rate.

In the comparative study of the four races the influence of yeast is not evident. Both control and experimental lines respond with increased division rate if the Paramecia have previously been in a starved condition. If food has been plentiful the rich diet of malted milk and yeast seems to have little effect.

Contrasting results were obtained with pituitary solution added to the hay infusion and malted milk basic substances. Suprarenal extract added to the basic fluid in which the *Paramecia* lived, caused an increase in the rate of division.

This experiment is to be considered as an indicator only. It was undertaken with the point in mind that if these solutions influenced the division rate of *Paramecia* a more extensive work would follow, *i. e.*, the bacterial content would be taken into consideration. It has been a generally accepted fact that the normal diet of *Paramecium* is composed of various kinds of bacteria and that in their absence growth does not take place, and even maintenance is impossible. In 1908 Jennings said, "To make the conditions of existence the same, it is not sufficient to attend merely to the basic fluid, the bacteria must also be the same."

Hargitt and Fray (1917) elaborated on previous methods of determining the bacterial content and they proved that *Paramecium* can live in media which have undergone sterilization provided at least one sort of bacteria remains in the culture.

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